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Chronic Myeloid Leukemia as an Immunological Target

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Various clinical observations have implicated T cells in the control of chronic myeloid leukemia (CML). These observations have in recent years been supported by laboratory results indicating the presence of CML-specific T cells in the lymphocyte repertoire of both normal healthy individuals and disease-bearing patients. Both MHC-unrestricted and MHC-restricted immune effector mechanisms are involved. Donor lymphocyte infusion has produced encouraging GvL effects. However, future adoptive immunotherapy may depend on the isolation and generation of leukemia-specific T cells. Although many proteins may potentially act as leukemia antigens in CML for MHC-restricted cytotoxicity, the bcr-abl fusion protein has been most extensively investigated. There is now much evidence to suggest that the bcr-abl junctional peptides are capable of eliciting both CD4 and CD8 responses in normal healthy donors and CML patients. Furthermore, the T-cell lines generated react with autologous or HLA-matched fresh CML cells, suggesting that the bcr-abl fusion protein can be processed in vivo so that the joining segment is bound to HLA molecules in a configuration and concentration similar to those of the immunizing peptide for antigen recognition by the antigen-specific T-cell receptor. These results also indicate that the bcr-abl junctional peptides may be used for immunotherapy of CML. Other strategies available for immunotherapy of CML include immunologically or genetically manipulated donor T-cell infusion, the use of cytokines, adoptive immunotherapy with leukemia-reactive T-cells expanded ex vivo, and immune gene therapy. Novel and rational immunotherapy may therefore play an important adjuvant role in future in the management of patients with CML. Am. J. Hematol. 54:61–67, 1997

Key words: chronic myeloid leukemia; T-cell response; immunotherapy

INTRODUCTION

Although bone-marrow transplantation (BMT) has improved the prognosis of patients with chronic myeloid leukemia (CML), only a small proportion of patients are suitable for BMT. Other patients are either too old or do not have an HLA-matched marrow donor. In those patients who undergo BMT, the 3-year disease-free survival is only around 65% [1]. Posttransplant mortality is due to infection, graft-vs.-host disease (GVHD), or leukemia relapse. Conventionally, the relapse rate following allogeneic BMT for CML in first chronic phase is around 10–15%, indicating the frequent presence of residual disease following high-dose chemoradiotherapy and the resistance of leukemia progenitor cells to chemoradiotherapy. Unfractionating the total body irradiation or increasing the dose of radiotherapy for pretransplant conditioning, although decreasing the leukemia relapse rate, did not result in improvement of overall survival because the lower disease relapse rate was balanced by an increase in death from radiotoxicity [2]. Therefore, other forms of therapeutic modalities are needed.

EVIDENCE FOR THE ROLE OF T LYMPHOCYTES IN LEUKEMIA CONTROL IN CML

Various clinical observations have implicated T cells in the control of CML. These observations include a lower incidence of leukemia relapse in patients who developed GVHD following BMT [3], regression of leukemia after

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infusion of donor T lymphocytes into patients who relapsed following BMT [4], and a much higher incidence of leukemia relapse (>50%) following allogeneic BMT for CML using T-cell-depleted bone marrow [5]. Therefore, the mechanisms by which allogeneic BMT produces a lower leukemia relapse risk than chemotherapy alone are in part due to an antileukemic effector function mediated by the incoming donor graft. However, these observations also imply that graft-vs.-leukemia (GvL) effect is mediated by the same alloreactive donor lymphocytes which also cause GVHD, and that the antileukemic effect may be a manifestation of GVHD. If this is the case, there is only a very limited future for T-cell adoptive immunotherapy of leukemia, since enhancing the immune system would be without value in the autologous setting because of the absence of alloreactivity. Even following allogeneic BMT, the benefits of immunotherapy may be limited, since any improved survival attributable to an increase in GvL effect would be offset by an increase in mortality due to increased GVHD.

So, is there a distinct T-cell population which could mediate specific leukemia cytotoxicity without a global graft-vs.-host reaction? The best clinical scenario to suggest the presence of such a T-cell population is in CML patients treated with T-cell-depleted allogeneic BMT. The relapse rate is much higher than in transplantation performing using standard GVHD prophylaxis, even when controlled for similar degrees of GVHD, implying that the T-cell population responsible for GvL may be distinct from those alloreactive T cells causing GVHD, and suggesting that GvL and GVHD may be separable. Identification of these T cells may lead to novel adoptive immunotherapy to enhance GvL effects following BMT.

There are two T-cell effector mechanisms which may contribute to the control of leukemia in CML: major histocompatibility complex (MHC)-unrestricted and MHC-restricted cytotoxicity. The relative contribution of each mechanism towards leukemia control is still unclear. These two forms of cytotoxicity are mediated through different populations of lymphocytes and one may be more important than the other, depending on whether leukemia control occurs in the autologous or allogeneic setting. These two effector mechanisms may therefore be exploited for therapeutic use.

MHC UNRESTRICTED CYTOTOXICITY

Lymphocytes responsible for this mode of leukemic cell-killing consist of the natural killer (NK) and activated killer (AK) cells. These cells are mainly CD3-T-cell-receptor (TCR)-negative, but a proportion are CD3-TCR-positive. They are able to distinguish normal from leukemic progenitor cells, probably through a differential expression of cellular adhesion molecules on these two populations of progenitor cells. There is now sufficient laboratory evidence to support the cytotoxic potential of these cells for CML cells. NK cells isolated from donor bone marrow following allogeneic BMT are capable of lysing cryopreserved recipient CL cells in vitro [6], as well as inhibiting leukemia progenitor colony growth without affecting the normal granulocyte/monocyte colony-forming units (CFU-GM) [7]. These effector functions are mediated through either direct cellular cytotoxicity or cytokine release.

In the autologous setting, it is possible to generate cytokine-induced killer (CIK) cells from CML patients using a cocktail of interleukin (IL)-1, IL-2, interferon (IFN)γ, and anti-CD3 MoAb. These cells are able to lyse both autologous and allogeneic CML cells, and inhibit colony formation without affecting normal CFU-GM [8]. Leukemia cytotoxicity could be further enhanced without affecting normal CFU-GM by using activated-NK (A-NK) cells [9]. These A-NK cells are generated from adherent mononuclear cells activated with IL-2.

How then could we clinically exploit this mode of immunological mechanism in CML? Although it is possible for patients with CML to be administered intravenous high-dose IL-2 with or without specific NK stimulants such as IL-12 to augment NK activity and induce activated killer cells, the effectiveness of such an approach is questionable because of the high leukemia cell load. Experience with the use of IL-2 in acute myeloid leukemia suggests that T-cell immunotherapy is most optimal in the setting of minimal residual disease [10,11]. Therefore, a more effective way to immunotherapy of CML may be the administration of high-dose IL-2 following autologous transplant, using Philadelphia chromosome-negative stem cells harvested following high-dose chemotherapy and hemopoietic growth factor priming. The feasibility of such an approach has been demonstrated with acceptable toxicities in patients with other hematological malignancies [12]. The Philadelphia-negative autologous stem cells may also be further purged ex vivo with cytokines prior to reinfusion.

High-dose IL-2 could also be administered to augment NK and killer cell activity following allogeneic BMT, in order to lyse any residual leukemia cells remaining after the conditioning regimen. The timing of IL-2 administration may be crucial in this approach. Administration of IL-2 immediately following infusion of allogeneic bone marrow in a murine model not only increased GvL effect but also reduced GVHD [13], since NK and killer cells are capable of acting as veto cells to suppress alloreactivity [14]. Unfortunately, when such an approach was used in 7 patients receiving haploidentical donor marrow transplant, fatal GVHD was not prevented [15]. Later administration of IL-2 may also be possible but theoretically could increase GVHD and transplant-related mortality and morbidity. However, low or intermediate doses of IL-2 have been adminis-

tered to patients following allogeneic BMT with no apparent increase in GVHD [16,17]. Alternatively, donor NK cells may be expanded ex vivo using a cocktail of cytokines for adoptive immunotherapy following BMT in allogeneic settings.

MHC-RESTRICTED CYTOTOXICITY

MHC-restricted cytotoxicity is mediated by CD3-positive T cells, which may be either CD4 or CD8 in phenotype. These cells possess the α and B subunits of the T-cell receptor (TCR), and recognize leukemic cells which express leukemia-specific antigens in the form of unique peptides in association with MHC molecules. The leukemia antigen may be processed either by leukemic cells and presented in association with MHC class I molecules to generate a CD8 T-cell response, or by antigen presenting cells (APC) and presented in association with class II molecules to elicit a CD4 T-cell response. The resultant T-cell clone may contribute to leukemia control through cytokine release, apoptosis, or direct cytotoxicity. Presently the role of different lymphocyte populations in the antileukemic effect is still poorly defined and is likely to vary from one disease and from one patient to another. In CML, CD4 lymphocytes appear to be the effector cells primarily involved in leukemia cytotoxicity following allogeneic BMT, since donor marrow depleted of CD8+ cells did not impair the GvL effect [18].

Even when the antigen is appropriately processed and presented, a T-cell response can only occur if leukemia-reactive T cells are present in the T-cell repertoire. Antigen recognition by these T cells will result in T-cell activation and proliferation, with subsequent clonal expansion. If this is the case, it should be possible to identify these T cells in the peripheral blood of normal individuals and patients with CML. There is indeed much laboratory evidence demonstrating the presence of these T cells in patients with CML and in normal individuals.

Using a three-cell coculture system, autologous cytotoxic T lymphocytes (CTL) have been successfully generated from patients with CML [19]. These cells were CD3+, CD8+, and HLA-DR+, and they exhibited MHC-dependent cytotoxicity towards fresh autologous leukemic cells with minimal toxicity to normal bone marrow. More recently, Coleman et al. [20] used a coculture system to demonstrate the presence of MHC-restricted leukemia-reactive T cells in the peripheral blood of a patient with CML. These cells were normally anergic in vivo, probably related to the absence of appropriate costimulatory molecules on the leukemic cells. However, they became reactive after preincubation with high-dose IL-2. These T cells were able to proliferate, secrete IFNγ, and lyse cells upon rechallenging with fresh autologous leukemia cells. Leukemic cell recognition by the T cells appeared MHC-dependent. Both CD4 and CD8 responses were observed. The leukemia-reactive T-helper cells were present at a high frequency of 1:4,000 T cells, but a much lower frequency of 1:38,000 was observed in the cytotoxic T-cells, indicating that the immune reaction was mainly CD4-mediated. Both these studies [19,20] suggest that CML cells are able to elicit an autologous T-cell response, albeit an ineffective one, in patients with CML, indicating a potential reservoir of effector cells capable of controlling leukemia in disease-bearing patients.

Evidence relating to the presence of MHC-restricted cytotoxicity following allogeneic BMT is more extensive. Jiang et al. [21] found a surge of cytotoxic T cells reacting to host leukemia cells during marrow regeneration in patients undergoing allogeneic BMT for CML. These T cells showed a pattern of reactivity different to that against host PHA-stimulated lymphocytes, suggesting that the T cells were those distinctly mediating GvL. Furthermore, the cytotoxic T-lymphocyte precursor (CTLp) frequency to host leukemic cells correlated with clinical outcome, so that patients with a CTLp frequency of $<$1:400,000 relapsed, but those with a higher frequency remained in remission. Jiang et al. [6] also demonstrated a similar phenomenon in patients receiving donor buffy coat for relapsed CML following allogeneic BMT.

It has also been possible to demonstrate the presence of leukemia-reactive T cells following allogeneic BMT by selective T-cell depletion. Datta et al. [22] showed that it was possible to separate GvL from GVHD by challenging lymphocytes from a donor with PHA-stimulated lymphocytes from the recipient to generate an allore-active response, depleting the reacting T cells using CD25 antibodies. They found that the approach retained $>$75% of reactivity to CML cells but not PHA-stimulated lymphocytes, suggesting that this could be a strategy for enhancing GvL effect without inducing GVHD.

LEUKEMIA ANTIGENS IN CML

Antigens on leukemia cells which may be targeted include minor histocompatibility antigens (mHA) [23], overexpressed normal antigens, and leukemia-restricted antigens. An ideal antigen should not only be uniquely expressed by the malignant clone, but should also play a crucial role in the malignant process, so that the development of any subclone not expressing this antigen as a form of tumor escape mechanism by the malignant cells would hamper their neoplastic potential. Mutant oncogenes and fusion proteins arising following chromosomal translocations both appear to fit into this category. Other potential targets include normal antigens which are overexpressed by the malignant cells, e.g., MAGE and MART in the case of melanoma [24], and HER-2/neu protooncogene in ovarian and other epithelial tumors [25,26]. In CML, the candidate molecules for immune targeting include the bcr-abl fusion protein, mutant p53, and mutant

N-*ras*. If these neoantigens are processed and the unique peptides are presented on the cell surface in association with MHC molecules, they generate an immune response which may be CD4, CD8, or both. Both p53 [27] and mutant N-*ras* [28] have been demonstrated to be immunogenic to T cells from normal or disease-bearing individuals, suggesting that they could be potential targets for T-cell immunotherapy. However, p53 overexpression and N-*ras* mutations are rare events in CML. The bcr-abl fusion protein is, on the other hand, present universally among patients with CML. This fusion protein may be either b3a2 or b2a2 in nature, depending on the breakpoint junctions involved in the t(9,22) chromosomal translocation of the individual patients.

ABILITY OF bcr-abl FUSION PEPTIDES TO GENERATE CD4 RESPONSE

Early animal works involving immunization of Balb/c mice with fusion peptides suggested that a 12-mer b3a2 junctional peptide was able to elicit a CD4 T-cell proliferative response in vivo [29]. The resultant T-cell clone was able not only to respond to the original 12-mer peptide but also to peptide lengths of up to 22 mers, suggesting that a broad spectrum of joining-region peptides could be appropriately presented by class II MHC molecules. To investigate if the p210 protein could be processed by APC so that the joining segment is bound to class II molecules, T-cell clones were exposed to irradiated syngeneic APC and the bcr-abl proteins. A similar proliferative response was observed. These investigators [29] concluded that the bcr-abl protein may be processed by APC so that the joining segment is bound to class II molecules in a configuration and concentration similar to those of the immunizing peptide, to stimulate the antigen-specific TCR.

Synthetic peptides spanning the junction of the bcr-abl proteins have been shown to elicit a CD4 response in healthy normal individuals. Ten Bosch et al. [30] successfully used a 17-mer b3a2 fusion peptide to generate a bcr-abl-specific T-cell line which proliferated in response to the peptide from a healthy male donor. This T-cell response was HLA-DR2-restricted. HLA-DR11-restricted lymphocyte proliferative responses have also been observed in 2 of 6 normal donors using a 25-mer b3a2 junctional peptide [31]. These results therefore support the immunogenicity of the bcr-abl fusion protein in both murine models and normal human subjects to result in a CD4 proliferative response.

ABILITY OF bcr-abl FUSION PEPTIDES TO GENERATE CD8 RESPONSE

A prerequisite for the induction of CTL is the ability of a peptide to bind to class I molecules. Using antigen-processing defective cell lines, Cullis et al. [32] tested a panel of overlapping peptides corresponding to the junctional sequences of bcr-abl proteins and did not find any peptide binding to HLA-A2 and -B35 transfected mutant cell lines, indicating that the bcr-abl junctional peptides are unlikely to be presented to T cells in association with HLA-A2 and -B35. However, binding assays measured by the inhibition of standard radiolabelled peptides to purified HLA class I molecules showed that some b3a2 8–11-mer peptides were able to bind with high or intermediate affinity to the HLA-A3, -A11, and -B8 molecules [33], suggesting that potentially the b3a2 junctional peptide could bind HLA molecules to induce a CD8 CTL response.

Evidence for the generation of specific CD8 CTL towards bcr-abl junctional peptides in humans is accumulating. Bocchia et al. [31] successfully generated bcr-abl-specific CTL from normal donors and demonstrated that the CTL was able to induce killing of HLA-A3 matched leukemia cell line. Moreover, the CTL was also able to kill both autologous and allogeneic HLA-matched peptide-pulsed PMNC. Dermime et al. [34] used both b2a2 and b3a2 peptides to generate HLA-B8-restricted CTL using normal PMNC. These cells were able to kill autologous lymphoblastoid cells and a plasma cell line transfected with the HLA-B8 molecules and pulsed with the peptides. Furthermore, killing of HLA-matched fresh CML cells was achieved. Both these results therefore demonstrated that MHC-restricted bcr-abl-specific CTL are present in the T-cell repertoire of normal healthy donors. More importantly, they also showed the ability of these T cells to mediate killing of MHC-matched fresh CML cells and suggested the presence of T-cell epitopes which may be derived from or crossreactive with the bcr-abl proteins.

The possible clinical relevance of bcr-abl junctional peptides in leukemia-bearing patients has also been demonstrated. Greco et al. [35] used a b3a2 nanopeptide to generate HLA-A3-restricted CTL from the lymphocytes of HLA-A3-positive CML patients. They found that this was successful in some patients after a single round of in vitro stimulation, favoring the notion that the T cells were already primed in vivo. HLA-A2. 1-restricted CTL which were capable of lysing fresh autologous CML cells and K562 erythroleukemia cells transfected with the HLA molecules have also been successfully generated using junctional nanopeptides of the bcr-abl protein [36]. These results suggest that CTL recognizing the bcr-abl junctional peptides are present in the lymphocyte repertoire of patients with CML. So why do these patients still develop CML? The reasons for the failure of CTL in leukemia immunosurveillance remain speculative, but may be due to leukemia escape when immunosurveillance is temporarily impaired (by drugs or viral infections), failure to generate an effective T-cell response due to

low copy numbers of the bcr-abl peptide present on the leukemic cell surface, or lack of appropriate costimulatory molecules.

MHC-RESTRICTED T-CELL IMMUNOTHERAPY OF CML

There are a number of approaches which could be used in MHC-restricted T-cell immunotherapy of CML. These are summarized in Table I. First, the antileukemic effect of BMT may be enhanced by donor lymphocyte infusion. This has been carried out following allogeneic BMT for acute leukemia using T-cell-depleted donor marrow [37]. Administration of donor lymphocytes in such circumstances was associated with clinically significant GVHD, but decreased relapse rate. This approach, however, may only confer GvL similar to that achieved with unmanipulated BMT. More recently, careful titration of the doses of donor lymphocytes has been shown to produce an antileukemic effect without GVHD when donor lymphocytes were infused to patients relapsed following allogeneic BMT for CML [38]. Although this approach was primarily used for patients with relapsed CML, it may be possible to use such a strategy in high-risk patients as an adjuvant immunotherapy following BMT. The risk of GVHD from donor lymphocytes may also be reduced by using herpes simplex thymidine kinase-transduced donor lymphocytes so that these lymphocytes can be eradicated, if necessary, by administration of ganciclovir [39].

Secondly, selective T-cell depletion of donor lymphocytes can be carried out when considering allogeneic adoptive immunotherapy to enhance the GvL effect of donor marrow. So far, results of allogeneic BMT using selective T-cell depletion with CD8 [18] and CD6 [40] antibodies have produced a low incidence of GVHD. Furthermore, CD8 depletion did not appear to affect the GvL effect [41] in patients receiving donor lymphocyte infusion for relapsed CML following allogeneic BMT. This approach may therefore be used with minimal risks of GVHD, while still preserving GvL effect.

Thirdly, following autologous or allogeneic BMT, the antileukemic effect of BMT may be enhanced by adoptive immunotherapy with T cells generated and expanded ex vivo using synthetic bcr-abl junctional peptides. In the autologous setting, this strategy may also be enhanced by the peritransplant administration of cyclosporin A with or without lymphokines, which would induce autologous GVHD. The disadvantage of this approach is that, unless a full panel of overlapping peptides and potential leukemia antigens are used, only one T-cell epitope and one potential leukemia antigen are targeted. This may not provide an optimal leukemia cytotoxicity. Furthermore, even though ex vivo generation and expansion of T-cell clones have been adequately successful to allow its clinical use for Epstein-Barr virus [42] and cytomegalovirus infection [43], the task involved should not be underestimated. This would limit its widespread use.

TABLE I. Approaches to T-Cell Immunotherapy of CML

1. Donor lymphocyte infusion: Suitable for patients who have undergone allogeneic BMT. Risk of GVHD may be reduced without compromising GvL effects by careful titration of lymphocyte dose or by using herpes simplex thymidine kinase-transduced T-cells.
2. Selective T-cell depletion of donor lymphocytes: Suitable for patients who have undergone allogeneic BMT. CD8-depletion in CML has been associated with low incidence of GVHD without apparent increase in disease relapse.
3. Adoptive immunotherapy using T cells generated and expanded ex vivo using synthetic bcr-abl junctional peptides: Suitable for both autologous and allogeneic settings. May be technically difficult to generate adequate T cells for in vivo use. Leukemia cytotoxicity may be suboptimal, since only one T-cell epitope and one potential leukemia antigen are targeted.
4. bcr-abl peptide vaccination: Suitable for both autologous and allogeneic settings. Involves administration of large amounts of peptide intravenously to disease-bearing individuals so that the peptide may compete for binding to the MHC molecules to induce a T-cell response. Technically easy, but may suffer similar disadvantages as approach 3.
5. Polyclonal CML-reactive T-cell adoptive immunotherapy: Involves molecular detection, isolation, and expansion ex vivo of clonally expanded T cells for infusion. This approach is cumbersome, but may include a broad range of polyclonal T cells reactive with autologous leukemia cells, without the need to identify leukemia antigen.
6. Molecular immunotherapy: Involves genetic manipulation of leukemia cells, e.g., transduction of costimulatory molecules or T-cell stimulatory molecules, to enhance immunogenicity of leukemia cells.

Fourthly, instead of generating leukemia-specific T cells ex vivo, peptide vaccination may be used. This would involve the administration of large amounts of peptide intravenously to disease-bearing individuals so that the peptide may compete for binding to the MHC molecules and induce a T-cell response. This is an attractive option, since it is less laborious, and the presentation of peptides to T cells may be enhanced by using ex vivo-generated dendritic cells [44]. This strategy, however, suffers the same disadvantages of targeting only one epitope and one leukemia antigen.

Fifthly, it may be possible to isolate and expand polyclonal CML-reactive T cells from patients with CML and use them as adoptive immunotherapy in the setting of minimal residual disease, e.g., following autologous transplant using Philadelphia chromosome-negative peripheral blood stem cells. Advances in molecular immunology and understanding of the organization of the TCR have allowed the specific analysis of the T-cell repertoire at a clonal level. Furthermore, it is now possible to identify clonally expanded T cells using reverse transcriptase/polymerase chain reaction of the TCR Vβ genes, followed by accurate analysis of PCR products for a distortion in the normal length distribution profile by fluorescence gene scanning. Analysis of the TCR Vβ repertoire in squamous-cell carcinoma [45,46], renal-cell carcinoma

[47], CML [48], and chronic lymphocytic leukemia [49] identified either a restricted TCR Vβ repertoire or clonally expanded T cells, indicated by recurring TCR Vβ transcripts. Presence of any clonally expanded cells may therefore by positively selected using monoclonal antibodies towards the TCR Vβ family and tested for their antileukemic activity against autologous leukemic cells. T cells showing leukemia-specific activity may be expanded for in vivo use. Though cumbersome, this approach will include a broad range of polyclonal T cells reactive with autologous leukemic cells, without the need to identify the exact leukemia antigens involved in the T-cell response.

Finally, genetically modified autologous CML cells may be used to augment the T-cell response. It is likely that T cells present in most CML patients are anergic to autologous CML cells because of the lack of appropriate costimulatory molecules such as the B7.1 molecules on leukemic cells. Therefore, T-cell recognition of leukemic cells only leads to partial T-cell activation, with subsequent development of anergy. Coleman et al. [20] demonstrated that this anergic state may be reversed by in vitro preincubation of T cells with high-dose IL-2. If this is the case, the immunogenicity of and T-cell reactivity to CML cells may be enhanced by retroviral transfer of both the IL-2 and B7.1 genes into the CML cells. T-cell activation may be achieved using genetically manipulated irradiated leukemic cells, which are now equipped with costimulatory molecules and IL-2 as stimulator, and any T-cell clones resulting will react with the native CML cells, since T-cell recognition does not need either IL-2 or costimulatory molecules. The advantage of this approach is that the response, which will probably be polyclonal, can be achieved without knowledge of the leukaemic antigen. Unfortunately, gene transfer efficiency is extremely low in CML (Lim, unpublished data) due to the low number of cells in the cell cycle.

CONCLUSIONS

CML appears an ideal and exciting immunological target. Although there are potentially many ways in which the immunological effector function may be enhanced in vitro, the optimal approach to exploit this clinically remains unknown. Further work and better understanding of basic leukemia immunosurveillance will help clarify the approach and provide the basis for novel T-cell immunotherapy in CML.

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REFERENCES

1. Clift RA, Buckner CD, Thomas ED, Bensinger WI, Bowden R, Bryant E, Deeg HJ, Doney KC, Fisher LD, Hansen JA, Martin P, McDonald GB, Sanders JE, Schoch G, Singer J, Storb R, Sullivan KM, Witherspoon RP, Appelbaum FR: Marrow transplantation for chronic myeloid leukemia: A randomised study comparing cyclophosphamide and total body irradiation with busulfan and cyclophosphamide. Blood 84:2036, 1994.
2. Appelbaum FR: The influence of total dose, fractionation, dose rate, and distribution of total body irradiation on bone marrow transplantation. Semin Oncol [Suppl] 20:3, 1993.
3. Weiden PL, Flournoy N, Thomas ED, Prentice R, Fefer A, Buckner CD, Storb R: Anti-leukemia effect of graft-versus-host disease in recipients of allogeneic marrow grafts. N Engl J Med 300:1069, 1979.
4. Kolb HJ, Mitteruller J, Clemms C, Holler E, Ledderose G, Brehm G, Heim M, Wilmanns W: Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. Blood 76:2462, 1990.
5. Hale G, Waldmann H: Control of graft-versus-host disease and graft rejection by T cell depletion of donor and recipient with Campath-1 antibodies. Results of matched sibling transplant for malignant diseases. Bone Marrow Transplant 13:597, 1994.
6. Jiang YZ, Cullis JO, Kanfer EJ, Goldman JM, Barrett AJ: T cell and NK cell mediated graft-versus-leukaemic reactivity following donor buffy coat transfusion to treat relapse after marrow transplantation for chonic myeloid leukaemia. Bone Marrow Transplant 11:133, 1993.
7. MacKinnon S, Hows JM, Goldman JM: Induction of graft-versus-leukemia activity following bone marrow transplantation for chronic myeloid leukemia. Blood 76:2037, 1990.
8. Scheffold C, Brandt K, Johnston V, Lefterova P, Degen B, Schontube M, Huhn D, Neubauer A, Schmidt-Wolf IGH: Potential of autologous immunologic effector cells for bone marrow purging in patients with chronic myeloid leukemia. Bone Marrow Transplant 15:33, 1995.
9. Silla LMR, Pincus SM, Locker JD, Glover J, Elder E, Donnenberg AD, Nardi NB, Bryant J, Ball ED, Whiteside TL: Generation of activated natural killer (A-NK) cells in patients with chronic myelogenous leukaemia and their role in the *in vitro* disappearance of BCR/abl-positive targets. Br J Haematol 93:375, 1996.
10. Lim SH, Newland AC, Kelsey S, Bell A, Offerman E, Rist C, Gozzard D, Bareford D, Smith MP, Goldstone AH: Continuous intravenous infusion of high-dose recombinant interleukin-2 for acute myeloid leukaemia—A phase II study. Cancer Immunol Immunother 34:337, 1992.
11. Meloni G, Foa R, Vignetti M, Guarini A, Fenu S, Tosti S, Tos AG, Mandelli F: Interleukin-2 may induce prolonged remissions in advanced acute myelogenous leukemia. Blood 84:2158, 1994.
12. Hamon MD, Prentice HG, Gottlieb DJ, Macdonald ID, Cunningham JM, Smith OP, Gilmore M, Gandhi L, Collis C: Immunotherapy with interleukin 2 after ABMT in AML. Bone Marrow Transplant 11:399, 1993.
13. Sykes M, Pearson DA, Szot GL: IL-2-induced GVHD protection is not inhibited by cyclosporine and is maximal when IL-2 is given over a 25 hour period beginning on the day following bone marrow transplantation. Bone Marrow Transplant 15:395, 1995.
14. Azuma E, Kaplan J: Role of lymphokine-activated killer cells as mediators of veto and natural suppression. J Immunol 141:2601, 1988.
15. Przepiorka D, Ippoliti C, Koberda J, Chan K-W, Khouri IF, Fischer HE, Huh YO, Escudier SM, Seong D, Davis M, Gajewski J, Vriesendorp HM, Champlin RE: Interleukin-2 for prevention of graft-versus-host disease after haploidentical marrow transplantation. Transplantation 58:858, 1994.
16. Soiffer RJ, Murray C, Gonin R, Ritz J: Effect of low-dose interleukin-2 on disease relapse after T-cell-depleted allogeneic bone marrow transplantation. Blood 84:964, 1994.
17. Robinson N, Sanders JE, Benyunes MC, Beach K, Lindgren C, Thomp-

son JA, Appelbaum R, Fefer A: Phase I trial of interleukin-2 after unmodified HLA-matched sibling bone marrow transplantation for children with acute leukemia. Blood 87:1249, 1996.
18. Nimer SD, Giorgi J, Gajewski JL, Ku N, Schiller GJ, Lee K, Territo M, Ho W, Feig S, Selch M, Isacescu V, Reichert TA, Champlin RE: Selective depletion of CD8+ cells for prevention of graft-versus-host disease after bone marrow transplantation: A randomised controlled trial. Transplantation 57:82, 1994.
19. Somasundaram R, Advani SH, Gangal SG: Propagation of cytotoxic effectors from chronic myeloid leukemia patients and cloning of cytotoxic T-cells. Cancer Immunol Immunother 27:183, 1988.
20. Coleman S, Fisher J, Hoy T, Burnett AK, Lim SH: Autologous MHC-dependent leukaemia-reactive T lymphocytes in a patient with chronic myeloid leukaemia. Leukemia 10:483, 1996.
21. Jiang YZ, Kanfer EJ, Macdonald D, Cullis JO, Goldman JM, Barrett AJ: Graft-versus-leukaemia following allogeneic bone marrow transplantation: Emergence of cytotoxic T lymphocytes reacting to host leukaemia cells. Bone Marrow Transplant 8:253, 1991.
22. Datta AR, Barrett AJ, Jiang YZ, Guimaraes A, Mavroudis DA, van Rhee F, Gordon AA, Madrigal A: Distinct T cell populations distinguish chonic myeloid leukaemia cells from lymphocytes in the same individual: A model for separating GVHD from GVL reactions. Bone Marrow Transplant 14:517, 1994.
23. Falkenberg JHF, Goselink HM, van der Harst D, van Luxemburg-Heijs SAP, Kooy-Winkelaar YMC, Faber LM, de Kroon J, Brand A, Fibbe WE, Willemze R, Goulmy E: Growth inhibition of clonogenic leukemic precursor cells by minor histocompatibility antigen-specific cytotoxic T lymphocytes. J Exp Med 174:27, 1991.
24. Pardoll DM: Tumour antigens: A new look for the 1990s. Nature 369:357, 1994.
25. Fisk B, Blevins TL, Wharton JT, Ioannides CG: Identification of an immunodominant peptide of HER-2/neu protooncogene recognised by ovarian tumor-specific cytotoxic T lymphocyte lines. J Exp Med 181:2109, 1995.
26. Disis ML, Gralow JR, Bernhard H, Hand SL, Rubin WD, Cheever MA: Peptide-based, but not whole protein, vaccines elicit immunity to HER-2/neu, oncogenic self-protein. J Immunol 156:3151, 1996.
27. Tilkin AF, Lubin R, Soussi T, Lazar V, Janin N, Mathieu MC, Lefrere I, Carlu C, Roy M, Kayibanda M, Bellet O, Guillet J, Paillerets BB: Primary proliferative T cell response to wild-type p53 protein in patients with breast cancer. Eur J Immunol 25:1765, 1995.
28. Jung S, Schluesener H: Human T lymphocytes recognise a peptide of single point-mutated, oncogenic *ras* proteins. J Exp Med 173:273, 1991.
29. Chen W, Peace DJ, Rovira DK, You S-G, Cheever MA: T-cell immunity to the joining region of $p210^{BCR-ABL}$ protein. Proc Natl Acad Sci USA 89:1468, 1992.
30. Ten Bosch GJA, Toornvliet AC, Friede T, Melief CJM, Leeksma OC: Recognition of peptides corresponding to the joining region of $p210^{BCR-ABL}$ protein by human T cells. Leukemia 9:1344, 1995.
31. Bocchia M, Korontsvit T, Xu Q, Mackinnon S, Yang SY, Sette A, Scheinberg DA: Specific human cellular immunity to BCR-ABL oncogene-derived peptides. Blood 87:3587, 1996.
32. Cullis JO, Barrett AJ, Goldman JM, Lechler RI: Binding of BCR/ABL junctional peptides to major histocompatibility complex (MHC) class I molecules: Studies in antigen-processing defective cell lines. Leukemia 8:165, 1994.
33. Bocchia M, Wentworth PA, Southwood S, Sidney J, McGraw K, Scheinberg DA, Sette A: Specific binding of leukemia oncogene fusion protein peptides to HLA class I molecules. Blood 86:2680, 1995.
34. Dermime S, Molldrem J, Parker KC, Jiang YZ, Mavroudis D, Hensel N, Couriel D, Mahoney M, Coligan JE, Barrett AJ: Human CD8+ T lymphocytes recognize the fusion region of BCR-ABL hybrid protein present in chronic myelogenous leukemia. Blood [Suppl] 86:158, 1995.
35. Greco G, Fruci D, Accapezzato D, Barnaba V, Nisini R, Alimena G, Montefusco E, Vigneti R, Tanigaki N, Tosi R: Two bcr-abl junction peptides bind HLA-A3 molecules and allow specific induction of human cytotoxic T lymphocytes. Leukemia 10:693, 1996.
36. Langlade-Demoyen P, Abastado JP, Even J, Gourru G, Leblond V, Binet JL, Kourilsky P: Cytotoxic T cell response against 210 kDA chimeric protein coded by the BCR-ABL fusion gene in chronic myeloid leukemia. Blood [Suppl] 86:525, 1995.
37. Naparstek E, Or R, Nagler A, Cividalli G, Engelhard D, Aker M, Gimon Z, Manny N, Sacks T, Tochner Z, Weiss L, Samuel S, Brautbar C, Hale G, Waldmann H, Steinberg SM, Slavin S: T-cell-depleted allogeneic bone marrow transplantation for acute leukaemia using Campath-1 antibodies and post-transplant administration of donor's peripheral blood lymphocytes for prevention of relapse. Br J Haematol 89:506, 1995.
38. MacKinnon S, Papadopoulos EB, Carabasi MH, Reich L, Collins NH, Boulad F, Castro-Malaspina H, Childs BH, Gillio AP, Kernan NA, Small TN, Young JW, O'Reilley RJ: Adoptive immunotherapy evaluating escalating doses of donor leukocytes for relapse of chronic myeloid leukemia after bone marrow transplantation: Separation of graft-versus-leukemia responses from graft-versus-host disease. Blood 86:1261, 1995.
39. Thiberghien P, Reynolds CW, Keller J, Spence S, Deschaseaux M, Certoux J-M, Contassot E, Murphy WJ, Lyons R, Chiang Y, Herve P, Longo D, Ruscetti FW: Ganciclovir treatment of herpes simplex thymidine kinase-transduced primary T lymphocytes: An approach for specific in vivo donor T-cell depletion after bone marrow transplantation? Blood 84:1333, 1994.
40. Soiffer RJ, Murray C, Mauch P, Anderson KC, Freedman AS, Rabinowe SN, Takvorian T, Robertson MJ, Spector N, Gonin R, Miller KB, Rudders RA, Freeman A, Blake K, Coral F, Nadler LM, Ritz J: Prevention of graft-versus-host disease by selective depletion of CD6-positive T lymphocytes from donor bone marrow. J Clin Oncol 10:1191, 1992.
41. Giralt S, Hester J, Huh Y, Hirsch-Ginsberg C, Rondon G, Guo J, Lee M, Gajewski J, Talpaz M, Kantarjian H, Fischer H, Deisseroth A, Champlin RE: CD8+-depleted donor lymphocyte infusion as treatment for relapsed chronic myelogenous leukemia after allogeneic bone marrow transplantation: Graft vs. leukemia without graft vs. host disease. Blood 86:4337, 1995.
42. Rooney CM, Smith CA, Ng CY, Loftin S, Li C, Krance RA, Brenner MK, Heslop HE: Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr-virus-related lymphoproliferation. Lancet 345:9, 1995.
43. Walters EA, Greenberg PD, Gilbert MJ, Finch RJ, Watanabe KS, Thomas ED, Riddell SR: Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. N Engl J Med 333:1038, 1995.
44. Young JW, Inaba K: Dendritic cells as adjuvant for class I major histocompatibility complex-restricted antitumor immunity. J Exp Med 183:7, 1996.
45. Lim SH, Thomas DW, Coleman S, Stephens P: T cell receptor Vβ repertoire of tumour-infiltrating lymphocytes in oral squamous cell carcinoma. Cancer Immunol Immunother 42:69, 1996.
46. Caignard A, Dietrich PY, Morand V, Lim A, Pannetier C, Leridant AM, Hercend T, Even J, Kourilsky P, Triebel F: Evidence for T cell clonal expansion in patients with squamous cell carcinomas of the head and neck. Cancer Res 54:1292, 1994.
47. Gaudin C, Dietrich PY, Robache S, Guillard M, Escudier B, Lacombe MJT, Kumar A, Triebel F, Caignard A: *In vivo* local expansion of clonal T cell subpopulations in renal cell carcinoma. Cancer Res 55:685, 1995.
48. Coleman S, Perera P, Fisher J, Hoy T, Burnett AK, Lim SH: Abnormal TCR Vβ repertoire in patients with chronic myeloid leukaemic. Br J Haematol 90:358, 1995.
49. Ferace F, Kremer F, Dietrich PY, Gaudin C, Angevin E, Courtier MH, Bayle C, Hercend T, Triebel F: T cell repertoire in patients with B-chronic lymphocytic leukemia: Evidence for *in vivo* T cell clonal expansion. J Immunol 153:4281, 1994.